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Efficacy of Plant Metabolites of Imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae)

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Abstract: The metabolism of the chloronicotinyl insecticide imidacloprid is strongly influenced by the method of application. Whilst in foliar application most of the residues on the leaf surface display unchanged parent compound, most of the imidacloprid administered to plants by soil application or seed treatment is metabolized more or less completely, depending on plant species and time. The present study revealed that certain metabolites of imidacloprid which have been described in crop plants are highly active against aphid pests in different types of bioassays. Some of these metabolites showed a high oral activity against the green peach aphid (*Myzus persicae*), and the cotton aphid (*Aphis gossypii*). The aphicidal potency of the metabolites investigated was weaker in aphid dip tests than in oral ingestion bioassays using artificial double membranes. The most active plant metabolite was the imidazoline derivative of imidacloprid. The LC_{50} values of this metabolite for *M. persicae* and *A. gossypii* in oral ingestion bioassays were in the lower ppb-range, i.e. 0·0044 and 0·0068 mg litre^{-1}, respectively. Most of the other reported metabolites showed much weaker activity. Compared to imidacloprid, the imidazoline derivative showed superior affinity to housefly (*Musca domestica*) head nicotinic acetylcholine receptors, while all other metabolites were less specific than imidacloprid. It seems possible that, after seed treatment or soil application, a few of the biologically active metabolites arising are acting in concert with remaining levels of the parent compound imidacloprid, thus providing good control and long-lasting residual activity against plant-sucking pests in certain crops. © 1998 SCI.

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1 INTRODUCTION

The chloronicotinyl insecticide imidacloprid is highly active against homopteran pests such as aphids, planthoppers and whiteflies, but is also excellent in controlling some chewing pests, e.g. certain beetles.[1–3] Imidacloprid acts as an agonist of the nicotinic acetylcholine receptor (nAChR).[4] However, in contrast to other insecticides acting on nAChR, e.g. nicotine, imidacloprid is highly specific to insect receptors, as studies on different nAChR preparations from insects and vertebrates have revealed.[5–7] Insecticides acting on nAChR are not common and have not been used as heavily as

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organophosphates, carbamates and pyrethroids in the past. Thus imidacloprid is a valuable tool in resistance management strategies to control homopteran pests resistant to the above-mentioned conventional insecticides.[8,9]

Owing to its systemic properties, imidacloprid is often used in soil (drench) application and seed treatment in a variety of field crops, though its efficacy towards different plant-sucking pests after foliar application is also very good. In most cases a soil application or seed treatment is recommended, thus providing good residual activity for several weeks.[10,11] In contrast to foliar application, where most of the remaining residue on the leaf surface is imidacloprid, the active ingredient is metabolized more or less completely after soil application or seed treatment, depending on plant species and time. The general metabolic pathway of imidacloprid in plants after spray and granular application has been described earlier.[12,13] Some of these metabolites are closely related to imidacloprid, and the aim of our study was to elucidate the aphicidal potency of these closely related metabolites in oral ingestion bioassays using two main aphid pests, the cotton aphid (*Aphis gossypii* Glover), and the green peach aphid (*Myzus persicae* Sulzer), and to measure the affinity of plant metabolites of imidacloprid to nAChR in head membrane preparations from the housefly (*Musca domestica* L.). Biologically active metabolites could act in concert with remaining levels of imidacloprid in the plant and may provide residual activity as long as or longer than would imidacloprid residues alone.

2 MATERIALS AND METHODS

2.1 Insecticides, metabolites and chemicals

Imidacloprid was technical grade of the highest purity available. The purity of the imidacloprid metabolites, numbered **1–7** (Fig. 1), was at least 97%. Imidacloprid and the plant metabolites of imidacloprid were provided by Bayer AG (Leverkusen, Germany). [^{3}H]Imidacloprid ($1{\cdot}25 \times 10^{15}$ Bq mol^{-1}) for receptor binding studies was synthesized and labelled as has been described elsewhere.[5] All other chemicals and organic solvents used were of analytical grade. Stock solutions of imidacloprid and metabolites were prepared in acetone, and diluted with sucrose solutions (150 g litre^{-1}) or aqueous 'Triton' X-100 (1 g litre^{-1}) when oral or contact activity was bioassayed, respectively.

2.2 Aphids

The green peach aphid (*M. persicae*), an insecticide-susceptible strain and reared in the laboratory since 1967 was maintained on chinese cabbage (*Brassica pekinensis* (Lour) Rapr) at 22–23°C, 60% relative humidity and L16 : D8 photoperiod. An insecticide-susceptible strain of the cotton aphid (*A. gosspyii*) was maintained in the greenhouse on cotton plants (*Gossypium hirsutum* L.) at 24°C, 60% relative humidity and ambient photoperiod.

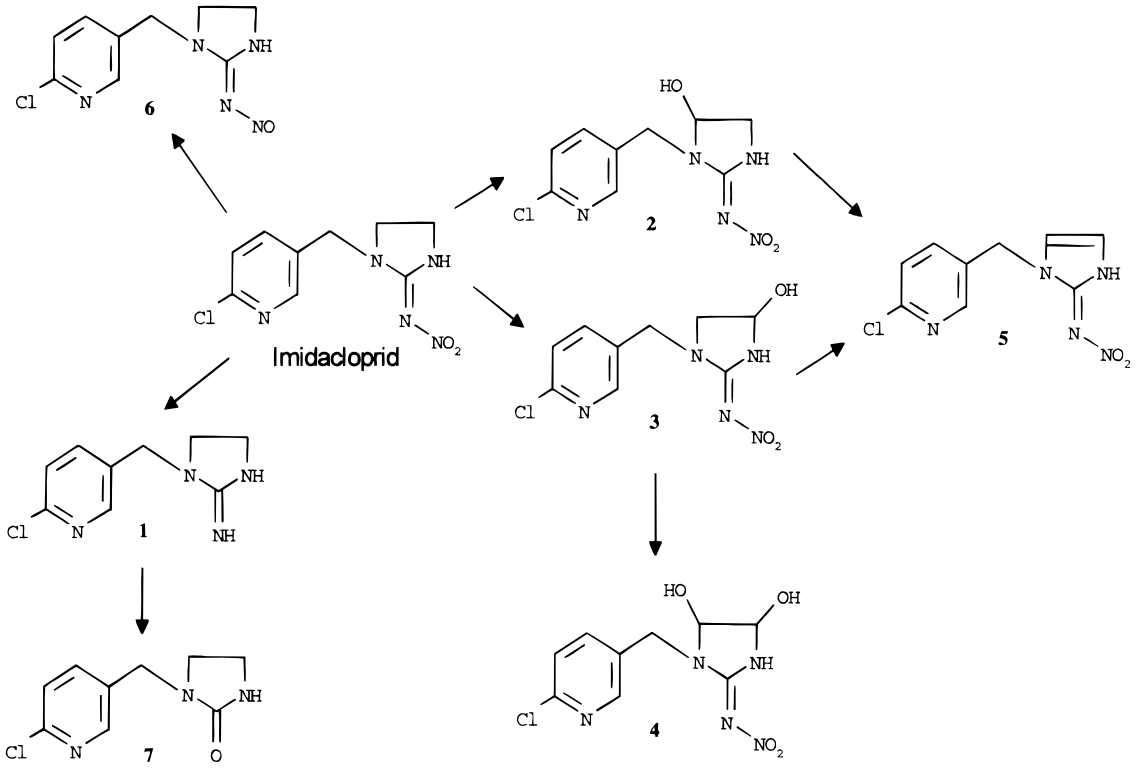

Fig. 1. Selected plant metabolites of imidacloprid investigated in this study.

2.3 Bioassays

All bioassays were performed in the laboratory at 21–22°C, 45–55% relative humidity and ambient photoperiod. All bioassays were repeated at least three times with two or three replicates of five or six different concentrations. The number of aphids in each bioassay ranged from 100 to 180. Lethal concentration values were computed from probit regressions using the computer program POLO PC (LeOra, Software, Berkeley, USA).

2.3.1 Feeding bioassay (sachet test)

The aphicidal potency of imidacloprid and its plant metabolites after oral ingestion was tested using a modification of the so-called sachet test described in detail elsewhere.[9,14] Acetonic stock solutions of the compounds were diluted in aqueous sucrose (150 g litre^{-1}); the acetone concentration in the bioassayed dilutions was less than 10 ml litre^{-1}. The prepared solution (0·4 ml) was pipetted between two layers of stretched 'Parafilm'® which formed the sachet. Groups of 10–15 aphids, which had been starved for 4 h prior the bioassay, were placed into small Petri dishes (diameter 2·8 cm) including an appropriate filter paper disc. The holding containers were sealed by stretching the prepared sachets across the top. A piece of yellow cellophane was placed over the artificial double membrane to enhance the feeding activity of aphids. Percentage mortality was determined after 48 h.

2.3.2 FAO dip test

The contact activity of some of the investigated metabolites was tested using a modified version of the FAO dip test.[15] Apterous adults of *Myzus* sp. were dipped for 10 s in insecticidal solutions containing 'Triton' X-100 (0·2 g litre^{-1}). After dipping, the aphids were transferred onto freshly excised cabbage leaves and the petioles were immersed in a small tube containing pure water. The leaves were placed in a plastic container of appropriate size and covered with a ventilated lid. Percentage mortality was scored 48 h post-dip.

2.4 Receptor binding assay

Binding of imidacloprid to housefly nAChRs was determined as described by Liu & Casida[5] with some minor modifications. Housefly heads stored frozen in liquid nitrogen were homogenized in a blender in sucrose solution (320 mM; 20 ml). After centrifugation for 10 min at 1200*g* the supernatant was filtered through five layers of cheesecloth and used directly for binding assays. The assay (total volume 1 ml) consisted of potassium phosphate buffer (0·1 M, pH 7·4; 0·7 ml) containing bovine serum albumin (binding buffer; 1 g litre^{-1}), homogenate (0·25 ml) and [^{3}H]imidacloprid (0·05 ml; 333 Bq = 0·266 pmol) in water containing methanol (16 ml litre^{-1}). Unlabeled imidacloprid was added in binding buffer containing up to 0·02 μl dimethylsulfoxide. After incubation for 60 min at 22°C, ice-cold binding buffer (3 ml) was added, followed immediately by filtration through prewetted Whatman GF/C glass fibre filters and rinsing with ice-cold binding buffer (2 × 3 ml). Bound radioactively was determined by scintillation counting of the filters. All values were measured in duplicates. pI_{50} values (-1 g M of the concentration of cold ligand displacing 50% of bound [^{3}H]imidacloprid) were calculated using a four-parameter logistic curve fitting program (GraFit, Erithacus Software Ltd.).

3 RESULTS AND DISCUSSION

The aphicidal potency of selected plant metabolites after oral ingestion was investigated using artificial double membranes. The test system produced reliable results and control mortality was in general less than 10%. All of the metabolites (**2** to **6**) which showed activity in the feeding bioassay described induced symptoms typical for compounds interfering with the insect nervous system, i.e. uncoordinated movement and tremor. The results revealed two plant metabolites (**5** and **6**) which were more active against *M. persicae* and *A. gossypii* than the parent compound imidacloprid itself (Tables 1 and 2). The most active metabolite was compound **5**, the imidazoline derivative (olefine metabolite) of imidacloprid. This metabolite showed a 16 times higher activity (based on $LC_{50(48\ h)}$ values) than imidacloprid on both aphid species after oral ingestion. The 95% fiducial limits indicate that the difference in efficacy between imidacloprid and metabolite **5** was significant. Another metabolite, the nitroso-derivative **6**, was also slightly more efficacious than imidacloprid, though not significantly so by considering the 95% fiducial limits which overlap with those for imidacloprid (Table 2). The hydroxy-substituted derivatives, compounds **2** and **3**, showed also considerable activity

TABLE 1
Efficacy of Plant Metabolites of Imidacloprid against *Myzus persicae* in Oral Ingestion Bioassays (48 h)

Compound	LC_{50} (*mg litre*$^{-1}$)	*FL 95%*	*Slope*
Imidacloprid	0·073	0·046–0·11	1·24
1	>10	—	—
2	3·2	0·42–20	0·85
3	0·53	0·20–0·97	1·1
4	5·9	1·1–37	1·2
5	0·0044	0·000 87–0·018	0·72
6	0·013	0·0090–0·017	2·1
7	>10	—	—

TABLE 2
Efficacy of Plant Metabolites of Imidacloprid against *Aphis gossypii* in Oral Ingestion Bioassays (48 h)

Compound	LC_{50} (*mg litre*$^{-1}$)	*FL 95%*	*Slope*
Imidacloprid	0·11	0·048–0·27	1·0
1	>10	—	—
2	1·1	0·37–3·1	0·94
3	0·85	0·24–2·8	1·2
4	9·3	1·4–54	1·0
5	0·0068	0·0031–0·014	0·70
6	0·048	0·021–0·11	0·84
7	>10	—	—

against *M. persicae* and *A. gossypii*. The 4-hydroxy-substituted compound **3** seems to be slightly more active against both species than compound **2** which is hydroxylated in position 5, though not significantly so. Even the dihydroxy metabolite **4** showed an LC_{50} value below 10 mg litre^{-1} for both species. Metabolites **1** (guanidine compound) and **7** (urea compound) were both inactive at the concentrations tested. In order to find out possible differences between oral activity and contact efficacy against *M. persicae*, some of the metabolites were also bioassayed using an aphid dip test. The aphid dip test revealed that, in contrast, to the results of the feeding bioassay, imidacloprid was superior to the olefine compound **5** (Table 3), perhaps indicating pharmacokinetic differences due to different physicochemical properties, e.g. the lipophilicity of compound **5** is lower and the water solubility higher than those of imidacloprid. The two monohydroxylated metabolites **2** and **3** were approximately 40 and 30 times less active in contact bioassays, respectively. This is in contrast to the results in the feeding bioassay with *M. persicae* where the 4-hydroxy-substituted metabolite **3** was just seven times less active than imidacloprid, indicating its high intrinsic potential, whereas the 5-hydroxylated compound **2** was more than 40 times less efficacious in the feeding bioassay than the parent compound, which coincides well with the results from the aphid dip bioassay.

TABLE 3
Contact Activity (Dip Test) of Some Plant Metabolites of Imidacloprid against *Myzus persicae* (48 h)

Compound	LC_{50} (*mg litre*$^{-1}$)	*FL 95%*	*Slope*
Imidacloprid	0·22	0·17–0·26	3·77
2	9·3	6·4–13	1·24
3	6·9	5·3–8·9	1·82
5	0·84	0·67–1·0	2·53

TABLE 4
Activity of Imidacloprid Metabolites as Inhibitors of [^{3}H]Imidacloprid binding to Nicotinic Acetylcholine Receptors in Housefly Head Membranes

Compound	IC_{50} (nM)
Imidacloprid	0·79
1	5000
2	5·0
3	25
4	630
5	0·25
6	n.d.
7	>10000

The biological efficacy in feeding bioassays on aphids correlates with the relative affinities of the metabolites towards the housefly nAChR (Table 4). The olefine metabolite **5** showed a somewhat higher affinity to the nAChR than imidacloprid, which was perhaps not surprising judging from its superior action in the feeding bioassay on both aphid species tested. In contrast to the bioassay results on *M. persicae* and *A. gossypii* where the 4-hydroxy metabolite **3** was slightly more active than metabolite **2** which is hydroxylated in position 5 of the imidazolidine ring, the receptor binding assay revealed the opposite, i.e. metabolite **2** showed a lower I_{50} value than metabolite **3**. It is not clear at present if nAChR preparations from other insect species such as aphids will show the same specificity as the housefly receptor. The lowest binding affinities, i.e. IC_{50} values of 5 μM, and >10 μM were measured with the biologically least-active guanidine metabolite **1** and the cyclic urea compound, metabolite **7**, respectively. The 4,5-dihydroxy metabolite **4** showed a poor affinity to the housefly nAChR, i.e. its IC_{50} value was more than 2500 times lower than the IC_{50} value for the most active metabolite, **5**. However, the biological efficacy of metabolite **4** on both aphid species was also 1000 times lower than that of metabolite **5**, indicating a rather good correlation between the binding data using tritiated imidacloprid as a radioligand in houseflies and insecticidal potency on aphids.

4 CONCLUSIONS

The conclusions which can be drawn from the results of this study are that perhaps in several cases of good long-lasting residual activity of imidacloprid after soil application or seed treatment, imidacloprid is not by any means the only compound which is responsible for the aphicidal effects observed. It is much more likely that imidacloprid *and* the orally active metabolites described here are acting in concert, i.e. a mixture of different metabolites including unchanged parent compound guarantees long-lasting insecticidal potency. It is unresolved so far if certain combinations of imid-

acloprid and its metabolites may act in a synergistic manner. Experiments with seed-treated cotton plants revealed that only 5% of the applied imidacloprid was taken up by the young plant and that, 27 days after sowing, approximately 95% of the parent compound was metabolized.[16] However, the residual activity is—depending on the aphid species considered—higher than 49 days on such plants, even though not all aphids show typical intoxication symptoms, but most of the individuals walk off treated plants due to the reported antifeedant action of imidacloprid.[11,17,18] Thus we could conclude that either the remaining amount of imidacloprid in such plants is still sufficient to control the aphids by virtue of its sub-lethal effects or that the appearance of certain metabolites in conjunction with low amounts of imidacloprid may provide the excellent control properties after seed treatment or soil application.

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